

STRUCTURE WATCH

Pumping iron

Bacteria have to resort to extreme measures to obtain iron, because it exists as ferric oxyhydroxide, a virtually insoluble form, in an oxidizing atmosphere. They secrete chelator molecules — siderophores — which bind ferric iron, and these complexes then bind to specific active transporter proteins in the outer membrane of Gram-negative bacteria. FecA from *Escherichia coli* is one of the most complex active iron transporters, and relies on the cytoplasmic membrane protein TonB to provide energy for this transport. In *Science*, Deisenhofer and colleagues now describe the crystallographic structure of FecA with and without bound ligand (in this case ferric citrate) at 2.5- and 2.0-Å resolution, respectively.

FecA is composed of three domains: a β -barrel domain that traverses the outer membrane, a plug domain located inside the barrel, and a periplasmic NH_2 -terminal extension. The authors found that FecA seems to have two gating mechanisms. One gating mechanism, previously seen in other active bacterial iron transporters, is provided by the plug domain, which prevents the direct passage of ferric citrate across the outer membrane. The second gating mechanism is provided mainly by the seventh and eighth extracellular loops of the β -barrel, which, after ligand binding, block the external pocket and ligand-binding site off from the external medium. These structural data have provided new insights into the gating mechanisms of TonB-dependent outer membrane proteins, and also enabled the authors to propose a mechanism for the energy-dependent transport of siderophores.

REFERENCE Ferguson, A. D. *et al.* Structural basis of gating by the outer membrane transporter FecA. *Science* **295**, 1715–1719 (2002)

Death machinery

Assembly of the apoptosome — a multiprotein ‘death’ complex — is a crucial step in the mitochondrial cell-death pathway, which requires apoptotic protease-activating factor 1 (Apaf-1), cytochrome *c* and dATP/ATP. The assembled apoptosome then binds to, and activates, procaspase-9, which can then activate executioner caspases such as caspase-3. The precise size, structure and number of subunits of the apoptosome are not known, and neither is the mechanism of procaspase-9 activation. However, in *Molecular Cell*, Akey and co-workers now provide the first three-dimensional structure of the apoptosome at 27-Å resolution, which was obtained using electron cryomicroscopy and molecular modelling techniques.

The structure shows a wheel-like particle with seven spokes radiating from a central hub. Each spoke is made up of a bent arm and a Y domain, with the latter having two lobes connected by a bridge. Using molecular modelling, Akey and colleagues were able to assign probable positions for Apaf-1 and cytochrome *c* within this structure, and to propose a model for apoptosome assembly, including a plausible role for cytochrome *c*. The authors also determined the structure of the apoptosome bound to a non-cleavable mutant of procaspase-9, which showed a dome-like feature on the central hub. This complex efficiently activated procaspase-3, indicating that procaspase-9 cleavage is not required to form an active cell-death complex.

REFERENCE Acehan, D. *et al.* Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. *Mol. Cell* **9**, 423–432 (2002)

B-CELL RESPONSES

AIDing diversity

Of humans, frogs, sharks and chickens, which is the odd one out? If you are interested in B cells, the answer is chickens — the first three animals use somatic hypermutation to diversify the variable (V) regions of their rearranged antibody genes, whereas chickens, together with pigs and rabbits, use gene conversion. Two groups have now shown that a putative RNA editing enzyme — activation-induced cytidine deaminase (AID) — essential for somatic hypermutation and immunoglobulin class-switch recombination is also required for gene conversion.

It is unclear why some species use somatic hypermutation and others use gene conversion. The processes are very different — somatic hypermutation involves the introduction of untemplated single base-pair alterations, whereas in gene conversion, new sequences are copied from nearby pseudogenes. However, it has been shown recently that when gene conversion is blocked in a chicken B-cell line, somatic hypermutation can occur instead, indicating that the two processes might be linked mechanistically.

The two new studies made use of the chicken B-cell line DT40, which undergoes gene conversion spontaneously. Hiroshi Arakawa and colleagues, reporting in *Science*, used a variant of this cell line with a frameshift mutation in its rearranged V segment that does not, therefore, express surface immunoglobulin (IgM). Gene conversion can repair the mutation, leading to the expression of surface IgM. The rate of reversion provides a quick and easy measure of gene conversion. AID was knocked out by gene targeting to create *AID*^{-/-} DT40 cells. Subclones were expanded for 18 days then analysed for surface IgM expression. The reversion rate in *AID*^{-/-} cells was reduced by at least 100-fold, and sequencing confirmed that this was due to an absence of gene conversion.

Reuben Harris and colleagues also generated *AID*^{-/-} cells, but in an IgM⁺ DT40 cell line. The cells were expanded for 40 days, then IgM^{low} cells were sorted and their V genes sequenced. None of the 89 *AID*^{-/-} cells analysed had undergone gene conversion, whereas gene-conversion events were detected in more than one-third of the *AID*^{+/+} cells.

These studies promote AID to the position of master controller of antibody gene modifications. The mechanisms of somatic hypermutation, gene conversion and class-switch recombination are unknown; however, it has been proposed that DNA breaks (double- or single-stranded) might be pivotal to all three processes. Harris *et al.* conclude by predicting, “it is likely that AID is involved in the formation of an initiating DNA lesion common to switch recombination, hypermutation and gene conversion”.

Jennifer Bell, Associate Editor, Nature Reviews Immunology

References and links

ORIGINAL RESEARCH PAPERS Arakawa, H., Hauschild, J. & Buerstedde, J. M. Requirement of the activation-induced deaminase (AID) gene for immunoglobulin gene conversion. *Science* **295**, 1301–1306 (2002) | Harris, R. S. *et al.* Immunoglobulin V gene conversion in a cultured B-cell line is dependent upon AID. *Curr. Biol.* 2002 February 11 (DOI 10.1016/S0960982202007170).

FURTHER READING Kinoshita, K. & Honjo, T. Linking class-switch recombination with somatic hypermutation. *Nature Rev. Mol. Cell Biol.* **2**, 493–503 (2001) | Martin, A. & Scharff, M. D. Antibody alterations. *Nature* **412**, 870–871 (2001)

